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Antifungal Activity of a Phytoterpenoid (AOS-A) Isolated from *Artabotrytis odoratissimus* on Spore Germination of Some Fungi

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Phytoterpenoid isolated from *Artabotrytis odoratissimus* inhibited spore germination of some plant pathogenic as well as saprophytic fungi e.g. *Alternaria alternata*, *A. solani*, *Cercospora* sp., *Curvularia maculans*, *C. pennisetti*, *Fusarium udum*, *Helminthosporium echinochlova*, *H. frumentacie*, *H. penniseti* and *Ustilago cynodontis*. In *Curvularia maculans* and *H. frumentacie*, spore germination was completely inhibited at 2000 ppm. However, *Curvularia maculans* and *C. pennisetti* showed considerable sensitivity to this chemical even at 500 ppm.

KEYWORDS: ***Artabotrytis odoratissimus*, Phytoterpenoid, Spore germination**

Fungal diseases of plants have always been one of the major constraints in crop production causing severe losses each year. Synthetic fungicides were used to manage some of the devastating diseases successfully. But, injudicious use of synthetic fungicides in disease control have resulted several hazardous effects on human as well as plant life due to their residual effect, which accumulate in human and animal bodies through polluted food chains. Use of chemical fungicides have been effective for controlling plant diseases but they induce resistance in fungal pathogens. Recent awareness of their negative effects warrant the use of eco-friendly and safe alternative methods for disease control. Some of the methods have currently been persuaded are biological control, genetic engineering for evolving disease resistant varieties and the induction of resistance by biotic and abiotic means (Lyon *et al*., 1995) and more importantly the use of biodegradable natural products.

Medicinal plants are known to contain several active components and some are quite effective against several phytopathogenic and saprophytic fungi (Maurya *et al*., 2001, 2002; Lyon *et al*., 1995; Basha *et al*., 2002; Sangita *et al*., 2005). A number of crude plant extracts have been tested by many workers for their efficacy against several plant pathogenic fungi *in vitro* as well as under field conditions (Chakarvarthy and Pariya, 1977; Asthana *et al*., 1982; Chaturvedi *et al*., 1987; Prithiviraj *et al*., 1996). On the other hand, several workers have recently used various active components against fungal spore germination *in vitro* (Maillard *et al*., 1987, 1989; Kobayashi *et al*., 1987; Singh *et al*., 1988, 1990; Prithiviraj *et al*., 1997a, b). Successful control of some plant diseases has been reported by active principles in the glasshouse (Remiers *et al*., 1993; Singh *et al*., 1995) as well as under field conditions (Prithiviraj *et al*., 1998). However, the use of plant products under field conditions is rare because of their small amounts in plants whose isolation in large quantity is usually cost prohibitive. Neemazal, a product of Neem (*Azadirachta indica*) and ajoene, a constituent of garlic (*Allium sativum*), have recently been used successfully against powdery mildew *(Erysiphe pisi*) of pea under field condition (Singh *et al*., 1995; Prithiviraj *et al*., 1998).

The active components that plants contain have been isolated in crude as well as in pure form, such as alkaloids, steroids, flavones, chalcone, phenolic acids and terpenoids. Terpenoids or isoprenoids are diverse group of natural organic compounds (hydrocarbons and hydrocarbons with oxygen) with common structural units and some of the general characteristics of lipids. The common structural unit is a five carbon (C_5H_8) molecule i.e. "isoprene". These compounds include hormones, some growth regulators, vitamins, terpentine and eubber etc (Loomis and Croteau, 1980).

Terpenoids have been reported to be antifungal against many phytopathogenic and saprophytic fungi. The old expeller neem oil (*Azadirachta indica*) fractions having a mixture of six tetranortriterpenoids derived through solvent partitioning have been found to be antifungal against *Dreschlera oryzae*, *Fusarium oxysoprum* and *Alternaria tenuis*. Farther methanolic extract of neem oil, which is a mixture of triterpenoid inhibited spore germination of 13 plant pathogenic fungi (Govindachari *et al*., 1998). Antifungal activity of Nibidin (a mixture of a number of triterpenoids) from seed oil of neem has been reported against *Alternaria tenuis*, *Fusarium oxysporum*, *Helminthosporium nodullosum* and *Curvularia tuberculata* whereas

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Isomeldin and Nimonol reported against the groundnut leaf rust pathogen (Khan *et al*., 1974; Suresh *et al*., 1997). The extract obtained from the resinous exudate of the plants *Pseudoganaphalium cheiranthifolium*, *P. heterotrichum*, *P. rubustum* and *P. vira* containing two diterpenoids inhibited the mycelial growth and conidial germination of *Botrytis cinerea* (Cotoras *et al*., 2001). Arteanniuma b, a sesquiterpenoid from *Artemisia annua*, showed antifungal activity against human pathogen i.e., *Candida albicans* and four plant pathogenic fungi i.e., *Gaumannomyces graminis* var. *tritici, Rhizoctonia aracalis*, *Curvularia nivalis* and *Verticillium dahliae* (Tang *et al*., 2000).

In the course of our continuous search for antifungal activity of plant products, a phytoterpeniod AOS-A, isolated from *Artabotrytis odoratissimus*, was tested against spore germination of some fungi belonging to different genera.

Materials and Methods

The fungi. The fungi were isolated on PDA (peeled potato 250 g, dextrose 20 g, agar 20 g, distilled water 1000 *ml*) from their respective hosts collected from the Experimental Farm of Banaras Hindu University. The cultures were purified by single spore isolation technique on PDA slants and maintained on the same medium for further experiments. The spores of obligate fungi were directly picked up from their respective hosts.

The plant product. The plant *Artabotrytis odoratissimus* is an evergreen perennial shrub, distributed in tropical Africa and Asia. It is used in Indian system of medicine for the treatment of vomiting, biliousness and diseases of blood and heart. The leaves of the plant have been reported to contain an antifertility component whereas oil of the seeds of the plant is known to have antidandruff, antietching and antithrombotic properties.

The well-dried, ground seeds of *A. odoratissimus* (0.8 kg) were successively extracted with petroleum ether at room temperature by stirring magnetically. The extract obtained by filtration was re-extracted in a soxhlet for 12 h with n-hexane (5 *l*). The n-hexane extract was concentrated to 0.5 *l* and left over night under room temperature. A residue was obtained. The hexane extract was then chromatographed over silica gel column eluting with solvent of increasing polarity. The collected eluents were monitored through TLC at every stage for their homogeneity. The similar eluents collected from hexane ($CHCl_3$-MeOH, 9 : 1 v/v) were mixed together and upon crystallization formed a mixture of four sesquiterpenes. Further column chromatography gave AOS-A. It exhibited $[\alpha]^{24}_{D}$ of 161~263°. Its molecular formula was determined as $C_{22}H_{46}O$ from the mass spectrum. It exhibited IR ν_{max}:

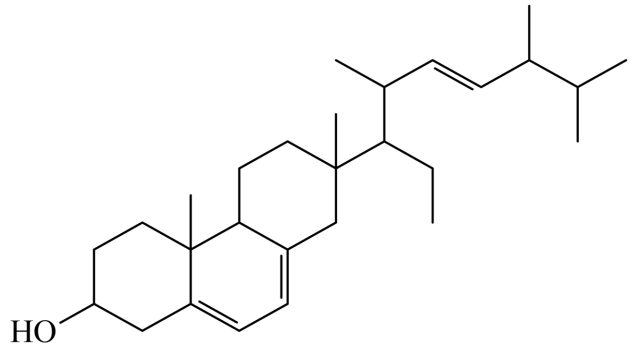

Fig. 1. Ergosta-5, 7, 22-triene-3-ol (AOS-A).

3500 (OH), 1380. 1365, 968; $EMS_{M|Z}$ (rel. int): $398^{[m]+}$ $^{[12]}$, 380 $[M\text{-}H_2O]^{+(67)}$, 365 $[M\text{-}H2O\text{-}Me]^{+271}$ $[C_{19}H_{29}O]^{+(22)}$: HRMS $_{M|Z}$: 398, 3529 for $C_{28}H_{29}O$. The ^{1}H-NMR spectrum of compound AOS-A ($C_{28}H_{46}O$) showed the presence of two tertiary methyl and four secondary methyl groups. One proton (δ - 3060, m) was attached to the oxygen bearing carbon and three protons (δ - 5.16, 3H, m) were attached to a double bond. Thus, suggesting AOS-A as an Ergosta- 5, 7, 22-triene-3 ol (Fig. 1).

Stock solution (5000 ppm) was prepared by dissolving 25 mg of chemical initially with a few drop of methanol: chloroform solution in a test tube. After the chemical was completely dissolved, approximately 4 *ml* of distilled water was added. The methanol:chloroform solution was then evaporated on a water bath. The required concentration (500, 1000, 1500, 2000 ppm) of chemical was prepared from the stock solution by diluting it with distilled water.

Antifungal activity. A drop (30~40 μl) of chemical solution was placed on a grease free glass slide. Fungal spores (200~300) were mixed in solution with the help of a sterile inoculation needle. Spores of *Ustilago cynodontis* and *Cercospora* sp. were directly picked up from diseased plant and mixed in drop of solution. The slides were later placed in a moist chamber made by placing two sterilized moist filter paper on lid and bottom of the Petriplates. The spores were than incubated at 25 ± 2°C for 24 h for germination. The germination of the spores was observed after staining with cotton blue prepared in lactophenol under binocular light microscope (Nikon, Japan). The experiment was conducted in triplicate.

Results and Discussion

The effect of the phytoterpenoid AOS-A on spore germination of some plant pathogenic fungi was observed. The sensitivity of different fungi to this compound varied considerably. Among the test fungi, four fungi, viz., *Alternaria solani*, *Curvularia maculans*, *C. pennisetti* and *Helminthosporium frumentacie*, were highly susceptible as significant inhibition of spore germination was observed

Table 1. Effect of a phytoterpenoid AOS-A isolated from *Artabotrytis odaratissimus* on spore germination of some fungi

Fungus	Host	Concentration (ppm)					CD
		0.00	500	1000	1500	2000	
		Percent germination					
Alternaria alternata	*Capsicum annum*	78.45	67.72	63.80**	32.12**	10.15**	13.34
Alternaria solani	*Solanum tuberosum*	86.99	82.12**	75.89**	50.77**	11.93**	4.08
Cercospora sp.	*Abelmoschus esculentum*	68.55	61.73	61.94	53.17**	35.73**	12.77
Curvularia maculans	*Musa paradisica*	85.05	25.47**	13.54**	0.68**	0.00	14.97
Curvularia pennisetti	*Pennisettum typhoids*	84.66	31.73**	21.13**	15.33**	6.33**	11.32
Fusarium udum	*Cajans cajan*	81.35	70.97	64.62	43.73	19.22	14.58
Helminthosporium pennisetti	*Pennisettum typhoides*	90.51	90.08	72.09	50.12**	1.96**	27.79
Helminthosporium echinochlova	*Echinochlova crusgalli*	86.99	78.34	77.85	.04**	12.36**	25.72
Helminthosporium frumentacie	*Echinoclova frumentum*	87.09	73.15**	69.19**	21.05**	0.00	12.98
Ustilago cynodontis	*Cynodon dectylon*	72.37	66.70	64.69	41.70	36.83	27.09

Value with ** is significantly different from its corresponding control value at $P \leq 0.01$.
CD, Critical Difference.

at 500 ppm. However, complete inhibition of spore germination was observed only in two fungi, viz., *C. maculans* and *H. frumentacie* at 2000 ppm. The spore germination of *H. pennisetti*, *C. pennisetti* and *Alternaria alternata* was also inhibited by 98.04, 93.67 and 89.85%, respectively, at this concentration. However, *Ustilago cynodontis* and *Cercospora* sp. were less sensitive as they showed only 36.63 and 35.73% spore germination, respectively, at 2000 ppm (Table 1).

There are numerous reports on active plant components showing antifungal activities (Sangita *et al*., 2005; Maurya *et al*., 2001; Singh *et al*., 2000). Many of them are formulated and the formulated products have also shown significant antifungal activity under field conditions (Singh *et al*., 1999). Similarly, antifungal activity of sunflower terpenoids was observed by Pitchman *et al*. (1990). The results from the present investigation indicates the antifungal nature of the phytoterpenoid AOS-A which showed significant inhibition in spore germination of a wide range of phytopathogenic fungi including biotrophs. The antifungal property of AOS-A was observed for the first time and looking into the high efficacy of the compound in inhibiting spore germination in the present investigation, it is considered as a potential antifungal agent for future application. However, detail studies regarding its mode of action and efficacy under field condition are needed to be carried out before its wide application.

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